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WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 7/04, A61K 37/02, 37/64

A1

(11) International Publication Number: WO 92/03474

(43) International Publication Date: 5 March 1992 (05.03.92)

(21) International Application Number: PCT/US91/05996

(22) International Filing Date: 22 August 1991 (22.08.91)

(30) Priority data:
572,671 24 August 1990 (24.08.90) US

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(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF INTERFERING WITH FORMATION OF α - ANTICHYMOTRYPSIN - β - PROTEIN COMPLEX AND SYNTHETIC PEPTIDES FOR USE THEREIN

(57) Abstract

A method of interfering with formation of a complex of α_1-antichymotrypsin and Alzheimer's β-protein and synthetic peptides useful in the method. The method and peptides are useful in preventing formation of such complexes in individuals.

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METHOD OF INTERFERING WITH FORMATION OF α-ANTICHYMOTRYPSIN-β-PROTEIN COMPLEX AND SYNTHETIC PEPTIDES FOR USE THEREIN

Description

Background

Alzheimer's disease is a degenerative disorder of the central nervous system that results in a progressive loss of memory and other intellectual functions, such as reasoning, orientation, and judgement (R. Katzman, Banbury Report 15: Biological Aspects of Alzheimer's Disease, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1983)). Alzheimer's disease occurs in sporadic and familial forms, and in the United States, affects about 600 people for every 100,000. A characteristic aspect of the neuropathology of the disease is the occurrence of proteinaceous deposits referred to as "amyloid" in the cores of brain lesions called neuritic or senile plaques, as well as in cerebral blood vessels. The "amyloid" deposits are generally defined as 6-10 nm protein filaments with certain staining properties (Abraham, C.R. et al., Cell, 52:487-501 (1988)).

Amyloid deposits are also found in the brains of aged humans, although not as extensively as in Alzheimer's disease. Further, Down's syndrome patients more than 30 or 40 years old invariably develop the symptoms and neuropathology characteristic of Alzheimer's disease.

One component of the amyloid deposits was identified as A4 amyloid or β-protein (β-protein) and is 42 amino acids long (Glenner, G.G and C.G. Wong, Biochem. Biophys. Res. Commun. 120:885-890 (1984)). This protein is apparently derived from a larger membrane-spanning precursor protein which is alternately spliced to yield several products (Selkoe, D.J., Science, 248: 1058-1060). These observations suggested that the amyloid deposits in Alzheimer's disease could result from abnormal expression or posttranslational modification or processing of a normal molecule. Also intriguing was the finding that the gene encoding the amyloid protein precursor is located on chromosome 21, suggesting a common cause for the the deposits observed in Down syndrome, caused by trisomy of chromosome 21, and Alzheimer's disease.

As mentioned above, some cases of Alzheimer's disease appear to be familial, and are inherited in an autosomal dominant fashion. Linkage analysis in four families pointed to a lesion on the long arm of chromosome 21 (St. George-Hyslop, P.H. et al., Science, 238:664-660 (1987)), which correlated well with the mapping data and similarities between Down syndrome and Alzheimer disease. Recently, hereditary cerebral hemorrhage with amyloidosis of Dutch origin was reported to be linked to the APP gene, and a point mutation in the coding region of the gene was identified (Van Broeckhoven, C. et al., Science, 248:1120-1122 (1990); Levy, E. et al., Science, 248:1124-1126 (1990)). Patients with this disease have a form of the β-protein in amyloid deposits in meningeal and cerebral blood vessels.

However, other studies reported linkage of familial Alzheimer's disease to a locus on chromosome 21 distinct from the amyloid precursor protein (APP) gene (Tanzi, R.E. et al, Nature, 329:156-157 (1987); Van Broeckhoven, C. et al., Nature, 329:153-155 (1987)). Furthermore, there was no evidence of duplication of the APP gene in cases of familial or sporadic disease. In fact, studies of some families reportedly indicate no linkage to chromosome 21 (Schellenberg, G.D., Science, 241:1507-1510, (1988). These data suggest that there may be genetic heterogeneity in the cause of inherited forms of Alzheimer's disease, and other locations for the disease gene have been proposed, such as chromosome 14 (Weitkamp, L.R., Amer. J. Hum. Genet. 35:443-453 (1983)).

Thus, other components of the proteinaceous deposits in Alzheimer's disease may also be of interest and may provide clues to the cause or progress of the disease. In fact, a second component of the amyloid deposits has been characterized as α_1-antichymotrypsin (ACT), which, interestingly, is located on chromosome 14. Abraham et al. reported the identification of the serine protease inhibitor ACT in amyloid deposits in Alzheimer's disease brain. (Abraham, C.R. et al., Cell 52:487-501 (1988).

Summary of the Invention

This invention relates to a novel class of synthetic peptides or peptide-like compounds which mimic a component of a specific complex between the Alzheimer's β-protein and ACT, and which are useful to interfere with

formation of the complex. The invention also relates to a method of treating an individual in whom such complexes form, resulting directly or indirectly in an abnormal condition or disease state, and particularly to a method of treating an individual with Alzheimer's disease. The synthetic peptides of the present invention, which inhibit complex formation between the Alzheimer's β-protein and ACT by binding to the β-protein or to ACT, can be administered to an individual in such a manner as to interfere with the ACT-β-protein interaction, and in sufficient quantity so as to have the desired effect (i.e., reduction of complex formation and the abnormal disease state).

Brief Description of the Drawings

Figure 1 is the amino acid sequence of Alzheimer amyloid β-protein (top row) and the active sites of the following five serine proteases: cytotoxic T cell protease; cathepsin G; mast cell protease; trypsin; and chymotrypsin.

Figure 2 is a graphic representation of the protease regulatory activity of Alzheimer amyloid β-protein.

Detailed Description of the Invention

The protease inhibitor α_1-antichymotrypsin (ACT) and the 42-aa β-protein are integral components of the brain amyloid deposits of Alzheimer's disease, Down's syndrome, and normal aging. This indicates that there is a special affinity between ACT and the β-protein, perhaps essential to amyloid formation. A basis for this association is

suggested by the similarity of the N-terminus of β-protein to the active site of serine proteases.

As described herein, in vitro experiments demonstrate that ACT and β-protein form a complex that reflects the specificity and stability of a protease-inhibitor interaction. These results suggest a model for the amyloid filament and a physiological function for the β-protein. They also make it possible to design or select synthetic peptides which can be introduced into cells in order to interfere with (reduce or prevent) binding of the two components of the complex. Such synthetic peptides include peptides and peptide-like compounds (e.g., modified or derivatized peptides) which interfere with interaction of ACT with β-protein, particularly peptides which "mimic" the active (or binding) site of serine proteases. Such peptides can be short peptides in which the amino acid sequence is sufficiently homologous with the sequence of the binding site of a serine protease or with the N-terminus of β- protein as shown in Figure 1, that they bind with ACT. Alternatively, such peptides can include, in addition to the sequence sufficiently homologous with the serine protease binding site or β-protein region, other amino acids (e.g., one or more amino acids at either or both ends of the binding site sequence). According to the method of the present invention, a compound of the present invention is introduced into an individual in such a manner that it interferes with formation of the ACT-β-protein complex. In the present method of interfering with complex formation, a compound is administered in sufficient quantity and by an appropriate route to have a therapeutic effect.

The following is a description of the demonstration that ACT and β-protein interact with specificity to form a stable complex in vitro and of the compounds which can be used to interfere with the interaction. Such compounds and the method by which they are administered are also described.

The present invention can be used in the treatment of individuals, such as those with Alzheimer's disease, in whom complex formation would otherwise occur.

Demonstration of Formation of ACT-β-Protein Complex

The extracellular amyloid filaments found in the plaques and blood vessels of Alzheimer's disease, Down's syndrome, and normal aging, contain two proteins, both intimately associated with the filaments - the 42 amino acid β-protein and the serine protease inhibitor, α_1-antichymotrypsin (Glenner, G.G. and C.W. Wong, Biochem. Biophys. Res. Comm., 122:885 (1984); C.L. Masters, et al., EMBO J. 4:2757 (1985); D.J. Selkoe, et al., J. Neurochem. 46:1820 (1986); D.J. Selkoe et al., Science, 235:873 (1987); Abraham, C.R. and H. Potter, Bio/technology 7:147 (1989); D.J. Selkoe, Ann. Rev. Neurosci. 12:493 (1989); B. Muller-Hill, Ann. Rev. Biochem. 58:287 (1989); Neve, R.L. and H. Potter, in Molecular Genetic Approaches to Neuropsychiatric Disease, J. Brosius and R. Fremeau, Eds. (Academic Press, San Diego, in press); C.R. Abraham, et al., Cell, 52:487 (1988); C.R. Abraham, et al., Neuroscience, 32:715 (1989)). Recently, it was found that the major component of the vascular amyloid in the Dutch variant of hereditary cerebral

hemorrhage with amyloidosis (HCHWA-D) is the β-protein, not cystatin C, as in the Icelandic version (HCHWA-I), although the angiopathy appears similar (J. Ghiso, et al., Proc. Natl. Acad. Sci. 83:2974 (1986); S.G. van Duinen, et al., Proc. Natl. Acad. Sci. 84:5991 (1987)). When brain sections from individuals with this disease were analyzed by immunolabeling, ACT was also found to be present (M.M. Picken, et al., Am. J. Pathol., 134:749 (1989)). In contrast, amyloid deposits found in other diseases do not contain either β-protein or ACT. The biochemical characteristics of ACT and the β-protein suggests a basis for this special association. First, ACT is a serine protease inhibitor that functions by acting as a pseudosubstrate and binding covalently to its target protease to form a long-lived complex (J. Travis, et al., Biochemistry, 17:5651 (1978)). Second, an inspection of the sequence of the β-protein reveals a region near the N-terminus which shows a striking homology to one segment of the active site of serine proteases, including the key serine amino acid (Figure 1) (E. Roberts, Neurobiol. Aging, 7:561 (1986); Travis, J. and G.S. Salvesen, Ann. Rev. Biochem, 52:655 (1983); G. Salvesen, et al., Biochemistry, 26:2289 (1987)). Thus, it seemed possible that ACT and β-protein might be able to form a complex by virtue of a protease inhibitor-like interaction and that this complex contributes to the stability of Alzheimer amyloid filaments. This hypothesis was tested, as described below. Results showed that, in fact, the β-protein is able to specifically bind stably to the inhibitory active site of ACT.

As described in Example 1, various synthetic peptides were tested for their effect on the inhibition of chymotrypsin by ACT in vitro.

Figure 2 shows the results of assessment of protease activity, carried out with the synthetic peptides. As shown in Figure 2, when the ACT/chymotrypsin molar ratio was approximately 1:1, the ACT inhibited over 90 percent of the chymotrypsin activity. However, when ACT was pre-incubated with an approximately four-fold molar excess of synthetic peptides corresponding to amino acids 1-12 or 1-28 of the β-peptide prior to the addition of chymotrypsin, the inhibitory activity of ACT was substantially reduced and the chymotrypsin reaction rate increased 2 to 8-fold. In contrast, pre-incubation with even a 10-fold molar excess of a peptide corresponding to amino acids 258-277 of the β-protein precursor (which shows no similarity to the active site of serine proteases), failed to interfere with ACT. These data indicate that peptides showing similarity to the region around the key serine in the active site of serine proteases (Figure 1), and in particular the Alzheimer amyloid, β-protein, are able to interfere with the inhibitory function of a serine protease inhibitor, ACT. The specificity of the interaction indicates that it is occurring at the inhibitory active site of ACT. The fact that ACT still shows a substantial ability, even in the presence of the peptide, to inhibit chymotrypsin, probably reflects the fact that the binding of a protease inhibitor to its target involves many contacts with amino acids in the full

protease active site which the small peptides naturally lack.

Assessment of the Stability and Specificity of Formation of the α_1-ACT-β-Protein Complex

Unlike true substrates that form a transient covalent intermediate with the protease through the hydroxyl group of its reactive serine, and then become cleaved as the bond breaks and the protease resumes its active state, protease inhibitors become cleaved, but only very slowly release their attachment to the protease (J. Travis, et al., Biochemistry, 17:5651 (1978)). Thus, the inhibitors essentially inactivate the protease in a suicidal, stoichiometric interaction. The fact that serine protease inhibitors form stable complexes with their target proteases and that a serine protease inhibitor, ACT, is an integral component of the insoluble Alzheimer amyloid deposits, suggests that this protein might be incorporated into the filaments through a stable inhibitor-protease interaction. Therefore, the stability of the interaction between α_1-antichymotrypsin and the β-protein was assessed.. As described in Example 2, radio-iodinated peptides corresponding to amino acids 1-12 and 1-28 of the β-protein and the unrelated segment 258-277 of the β-protein precursor were prepared, incubated in the presence of ACT under various conditions, and the mixture electrophoresed on SDS polyacrylamide gels. In the absence of ACT, the low M.W. peptides migrated rapidly at the dye font. However, in the presence of ACT, a radioactive band was generated at a position corresponding to a few thousand M.W.

larger than the ACT protein. Thus, the interaction first detected in Figure 2 between ACT and the β-protein can be sufficiently stable to resist denaturation by boiling in SDS and β-mercaptoethanol. The indication in Figure 2 that the ACT--β-protein interaction occurs at the protease inhibitory site of ACT was confirmed by the fact that the addition of chymotrypsin to ACT prior to the radioactive peptide prevented the formation of the ACT-peptide complex. Heat denaturation of ACT prior to the addition of the peptide also prevented complex formation.

The specificity of the α_1-ACT-β protein interaction was demonstrated by incubating the 1-28 peptide with other proteins (e.g., BSA, CPK, and CA), with which it failed to form stable complexes.

In sum, the work described indicates that the two components of the Alzheimer amyloid deposits--ACT and the β-protein--can associate in vitro to form an SDS-stable complex. The interaction is specific for both the peptide and the ACT protein, and likely occurs between the active protease inhibitor site of ACT and the N-terminus of the β-protein, which resembles the active site of serine proteases.

These results suggest a model for the structure of the Alzheimer amyloid filaments. The hydrophobic C-terminal portion of β-protein molecules making up the filament would be tucked into the interior, while the hydrophilic central segment (amino acids 12-28) would form the surface of the filament. The protease active site-related amino acids at the N-terminus would form an arm projecting from the

surface of the filament and available for binding by ACT. However, the latter seems at least possible, inasmuch as the β-protein alone can form filaments in vitro having a β-pleated sheet conformation (D.A. Kirschner, et al., Proc. Natl. Acad. Sci., 83: (1986); E.M. Castano, et al., Biochem. Biophys. Res. Comm., 141:782 (1986); D.A. Kirschner, et al., Proc. Natl. Acad. Sci., 84:6953 (1987)). However, these filaments can easily be solubilized and therefore must lack a key component or structural conformation characteristic of true Alzheimer amyloid filaments. It is possible that the binding of a β-protein core filament to the relatively protease-resistant ACT provides the required extra stability.

These results also suggest a potential biological function for the β-protein. Since the β-protein can competitively interact with the active site of a serine protease inhibitor in vitro, it might be expected to be able to play a similar role in vivo. By decoying protease inhibitors (including the Kunitz-type inhibitor in the β-protein precursor), the β-protein, though not itself a protease, would serve as a protease enhancer--effectively increasing proteolytic activity in its vicinity. Overproduction of the β-protein, as would result, for instance, from increased proteolytic degradation of the β-protein precursor, could thus lead to a further increase in protease activity, with progressively adverse consequences.

Thus, as described herein, it has been shown that, in vitro, a serine protease inhibitor (ACT) interacts specifically with a serine protease-like target protein(Alzheimer β-protein) at a region of

the latter which bears striking sequence homology to the active site of serine proteases, resulting in formation of a stable ACT-β-protein complex. The work described herein provides a reasonable molecular mechanism for formation of the insoluble protein filaments that comprise the amyloid deposits of Alzheimer's disease.

As a result of the discovery of the specific interaction between ACT and Alzheimer β-protein, it is possible to design or select compounds which are useful to interfere with (reduce or prevent) this ACT-β-protein interaction. The discovery has also made possible a method of preventing the interaction. The compounds and method are particularly useful in reducing formation of ACT-β- protein complexes in individuals in whom such complexes form and result, directly or indirectly, in an abnormal or undesirable condition or a disease state. For example, such compounds can be used to reduce (totally or partially) or prevent formation of ACT-β-protein complexes in individuals who have Alzheimer's disease or would, without appropriate treatment, develop Alzheimer's disease.

Compounds of the present invention can be used to interfere with binding of ACT and Alzheimer's β-protein, either by binding to ACT, thus preventing formation of the ACT-β-protein complex, or by bind- ing to β-protein, also preventing formation of the ACT-β protein complex. For example, a peptide corresponding to all or a portion of the amino acid sequence of a serine protease, such as all or a por- tion of the sequence of the Alzheimer's β-protein can be used. Alternatively, a synthetic peptide which

mimics the amino acid sequence of the inhibitory active site of ACT can be used to interfere with ACT-β-protein complex formation. This type of peptide will bind to the β-protein, resulting in the production of a synthetic peptide-β-protein complex and, in essence, will "tie up" β-protein, precluding the β-protein from interacting with ACT.

The peptide compounds of the present invention may have additional effects resulting from their influence, direct or indirect, on the protease inhibitor function of ACT. ACT is a serine protease inhibitor and Alzheimer's β-protein has been shown to inhibit ACT's ability to inhibit a serine protease (i.e., chymotrypsin). Thus, because it decoys protease inhibitors, β-protein, although not itself a protease, enhances protease activity by effectively increasing proteolytic activity (i.e., by reducing ACT's ability to exhibit serine proteases).

This view of the interaction between ACT and the β-protein suggests alternate outcomes from administration of the two types of peptide. In the case where the peptide binds to ACT to inhibit complex formation, the peptide may have an inhibitory effect on ACT, resulting in enhancement of protease activity, in a manner similar to that suggested for the β-protein itself. In the case in which the synthetic peptide mimics the inhibitory active site of ACT and binds to the β-protein, one might expect the opposite outcome. That is, the peptide would complex with the β-protein, preventing its interaction with ACT, and thus blocking the postulated protease enhancing effect of the

β-protein. ACT then would be left free to exercise its function as a protease inhibitor.

The discovery described herein also makes it possible to identify ACT-β-protein complexes in tissue obtained from an individual, such as in biopsy tissues obtained from an individual suspected of having abnormally high levels of the complexes. This can be used, for example, to determine the presence or absence and, if desired, the quantity of ACT-β-protein complexes in brain tissue obtained at autopsy.

A synthetic peptide of the present invention can be administered in a physiologically acceptable carrier (e.g., an appropriate buffer or physiologic saline solution). It can be administered by any route via which it is possible to deliver a therapeutically effective quantity or dose to the individual in a form available to have the desired effect (reduction of complex formation). For example, a synthetic peptide of the present invention can be administered parenterally (e.g., intravenously or intramuscularly) in a composition which protects the peptide from degradation.

A synthetic peptide of the present invention can be made using known techniques, such as recombinant/genetic engineering techniques or chemical synthesis.

EXAMPLE 1 Assessment of the Protease Regulatory Activity of Alzheimer β-Protein

Various synthetic peptides were tested for their effect on the inhibition of chymotrypsin by ACT in vitro. The synthetic peptides tested were:

1. a peptide corresponding to amino acids 1-28 of the N-terminal portion of the Alzheimer β protein (See Figure 1).
2. a peptide corresponding to amino acids 1-12 of the N-terminal portion of the Alzheimer β protein (See Figure 1, first 12 amino acids); and
3. a control peptide corresponding to amino acids 258-277 of the Alzheimer β-protein precursor, which does not show any similarity to the serine protease active region sequence.

The activity of chymotrypsin was measured by cleavage of the chromogenic substrate Succinyl-Ala-Ala-Pro-Phe-nitroanilide. Results showed that chymotrypsin activity is reduced about 90 percent in the presence of an approximate equal molar concentration of ACT. If a four-fold molar excess of either of the two synthetic peptides from the N-terminal portion of the β-protein (corresponding to amino acids 1-28 and 1-12 respectively) are added to ACT prior to the protease assay, they interfere with the inhibitory activity of ACT. In contrast, the control peptide corresponding to amino acids 258-277 of the β-protein precursor does not modulate ACT's ability to inhibit chymotrypsin.

Results are shown in Figure 2. The data shown for each graph represent the average of four independent assays in which 0.5 (left) or 0.6 (right) μg of ACT were incubated for 2 min ± peptide

in 10 μl .1M phosphate buffer, pH 7, at 20°C prior to the addition of .3 μg of chymotrypsin. After a further incubation of 2 min, the reaction volume was increased to 0.8 ml, 15 μl of 2 mg/ml substrate was added and the reaction followed at OD 405 for five minutes. The graphs compare the relative slopes of the reaction curves (all straight lines) normalized to the reaction rate of chymotrypsin alone. The peptides did not have any independent effect on chymotrypsin, nor did they show protease or esterase activity of their own.

EXAMPLE 2 Assessment of the Stability of Interaction Between α-antichymotrypsin and β-protein

The ability of synthetic fragments of β-protein to inhibit ACT (Figure 2) indicates that at least a transient complex can form between the two proteins. The stability of such complexes can be demonstrated by incubating ACT with radiolabeled β-protein fragments under various conditions, and then analyzing the complex formation by polyacrylamide gel electrophoresis. In some experiments, a protein cross-linking agent (DSS) was used to stabilize the complex prior to gel electrophoresis. However, even in the absence of cross-linking, a complex is formed which is stable to boiling in SDS and β-mercaptoethanol. The addition of an equal molar amount of chymotrypsin to ACT blocks the active site and prevents the complex formation. Denaturing the ACT protein by heat also prevents the complex formation. Immunostaining of the blotted protein with antibodies to α_1-antichymotrypsin indicated that neither

the heat nor the chymotrypsin treatment destroyed the ACT. Thin layer chromatography confirmed that the amount of chymotrypsin added to the ACT was not sufficient to digest the peptide.

CLAIMS

1. A method of interfering with formation of a complex which includes of a first member which is α_1-antichymotrypsin and a second member which is β-protein, comprising contacting one member of the complex with a synthetic peptide which binds specifically to the member, thereby preventing binding of the first member and the second member.

2. A method of interfering with formation of an α_1-antichymotrypsin-β-protein complex, comprising contacting α_1-antichymotrypsin with a synthetic peptide which mimics the active site of a serine protease and binds to α_1-antichymotrypsin, thereby forming a synthetic peptide-α_1-antichymotrypsin complex.

3. A method of interfering with formation of an α_1-antiehymotrypsin-β-protein complex, comprising contacting α_1-antichymotrypsin with a synthetic peptide having all or a portion of the amino acid sequence of Alzheimer's amyloid β-protein as represented in Figure 1.

4. The method of Claim 3 wherein the portion of the amino acid sequence of Alzheimer's amyloid β-protein as represented in Figure 1 is amino acids 1-12 or amino acids 1-28.

5. A synthetic peptide having all or a portion of the amino acid sequence of Alzheimer's amyloid β-protein as represented in Figure 1.

6. A composition comprising a synthetic peptide of Claim 5 and a physiologically acceptable carrier.

7. A method of interfering with formation of α_1-antichymotrypsin-β-protein complexes in an individual, comprising administering to the individual a synthetic peptide selected from the group consisting of: synthetic peptides which mimic the amino acid sequence of the active site of a serine protease and bind α_1-antichymotrypsin; and synthetic peptides which mimic the inhibitory active site of α_1-antichymotrypsin and bind β-protein.

8. A method of Claim 7 wherein the synthetic peptide which mimics the amino acid sequence of the active site of a serine protease has an amino acid sequence sufficiently homologous to all or a portion of the amino acid sequence of the Alzheimer's β-protein represented in Figure 1 that they bind to ACT.

9. A method of Claim 8 wherein the synthetic peptide which has an amino acid sequence sufficiently homologous to all or a portion of the amino acid sequence of the region of

Alzheimer's β-protein represented in Figure 1 that it binds to ACT is homologous with amino acids 1-12 of Figure 1 or is homologous with amino acids 1-28 of Figure 1.

FIGURE 1

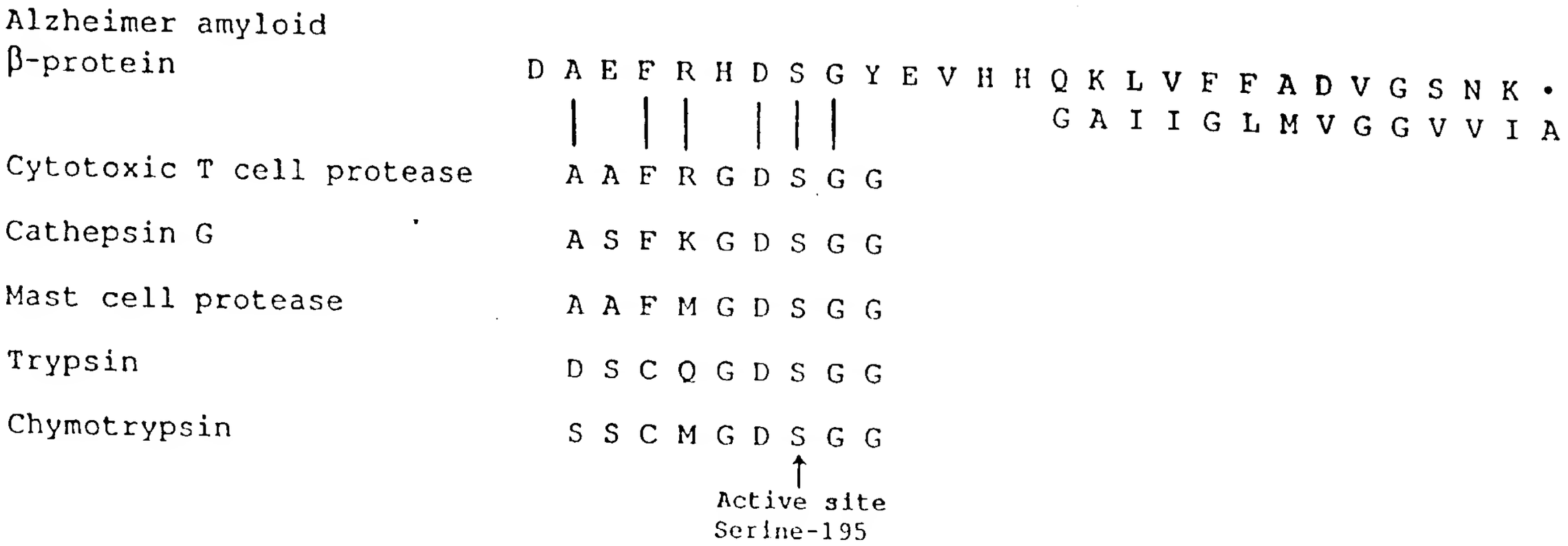

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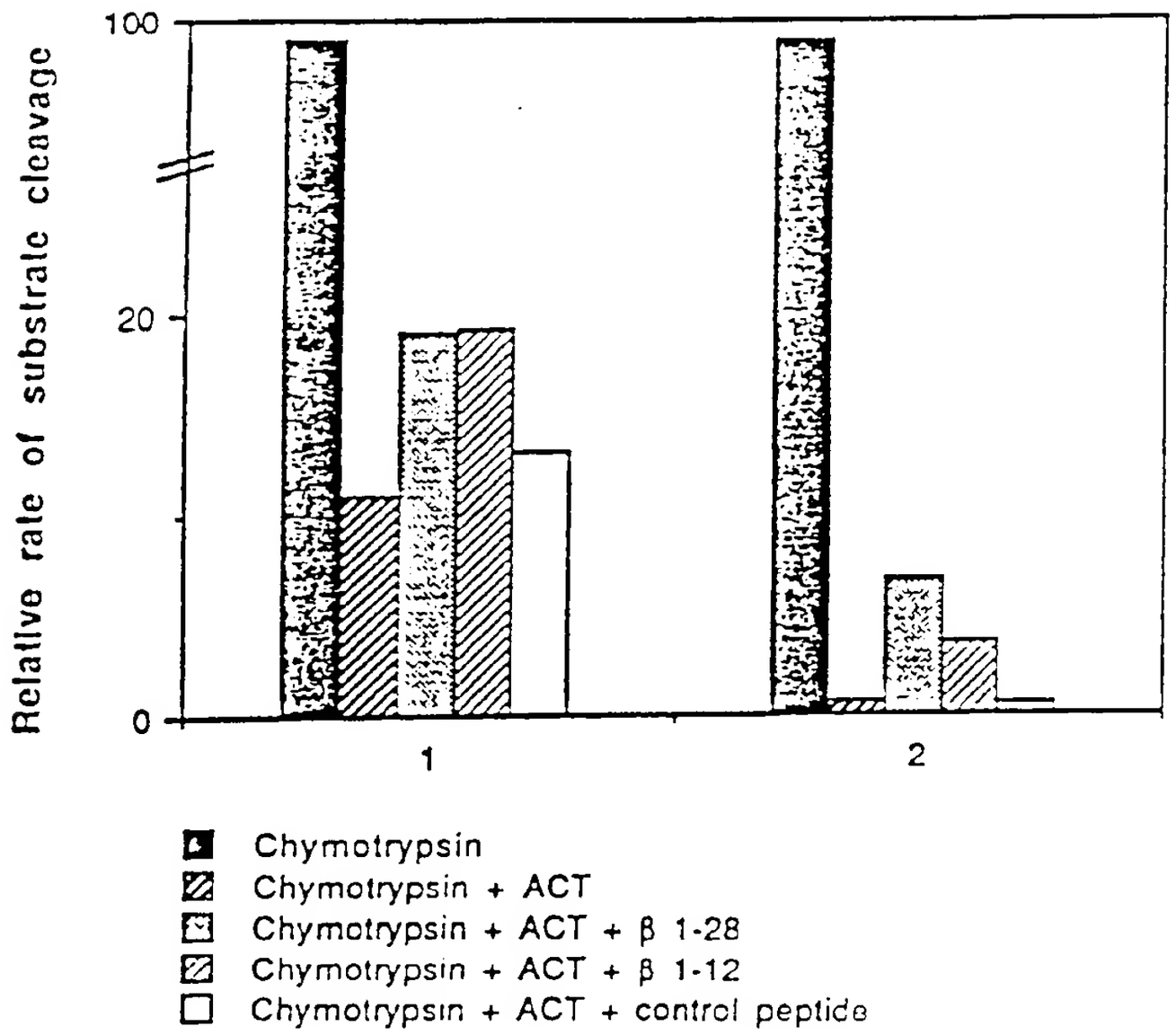
FIGURE 2


Alzheimer B-protein reduces the inhibition of chymotrypsin by ACT
Relative rate of substrate cleavage
100
20
0
1
2
Chymotrypsin
Chymotrypsin + ACT
Chymotrypsin + ACT + β 1-28
Chymotrypsin + ACT + β 1-12
Chymotrypsin + ACT + control peptide

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/05996

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 K 7/04 A 61 K 37/02 A 61 K 37/64

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Alzheimer's Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies, 15-20 July 1990, Toronto, Ontario, Canada, John Wiley & Sons Ltd (New York, US) H. Potter et al.: "The Alzheimer amyloid components alpha-1 antichymotrypsin and beta-protein form a stable complex in vitro", pages 275-280, see the whole article ---	1-9
X	Proc. Natl. Acad. Sci., volume 82, December 1985, Medical Sciences; "Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related", pages 8729-8732, see the whole article --- -/-	1-3,5-8

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13-01-1992	05 FEB 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Biochemistry, volume 2, no. 11, 20 March 1990, American Chemical Society (Washington, DC, US) K. Halverson et al.: "Molecular determinants of amyloid deposition in Alzheimer's disease: conformational studies of synthetic beta-protein fragments", pages 2639-2644, see the whole article -----	5

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely

Remark: Although claims 1-4 (partially) and 7-9 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition."

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6 4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers.

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest

☐ No protest accompanied the payment of additional search fees